Variation in Anthocyanin Production among Accessions and Cultivars of Rough Bluegrass (*Poa trivialis* L.)

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Introduction

Anthocyanins are plant secondary metabolites that are responsible for producing red and purple coloration in plant tissues (Dooner and Robbins, 1991). These plant pigments are associated with fall tree color, the color of many berries, a range of flower coloration, and are important in plant stress responses (Close and Beadle, 2003). Current research is also beginning to show that anthocyanins have significant potential in the development of new and emerging technologies. The ability of anthocyanins to act as photosensitizers has lent them the ability to serve as natural dyes in dye sensitized solar cells (Narayan 2012). These alternative methods to conventional solar energy technology are to be more cost effective and potentially more efficient than what is currently available (Hao et al., 2006).

Anthocyanins also possess a relatively high *in vitro* anti-oxidant capacity, which has caused many to speculate on their ability to serve as anti-oxidants when consumed as part of a daily diet (He and Giusti, 2010) . Anthocyanin anti-oxidant capacity has been theorized to increase heart health by protecting against low-density lipoprotein (LDL) oxidation; therefore, helping to decrease plaque accumulation within the arterial system (Pascual-Teresa et al., 2010). When exposed to a concentrated anthocyanin extract, malignant tumors have been shown to decrease in growth, and because of this anthocyanins are being considered chemopreventive and anti-cancer compounds (Lila 2004). Having such a large technological potential, the questions arises on how can the demand for these metabolites be met?

Biosynthesis of anthocyanins can either be a constituent process where the plant always produces some amount of the compound, or they can be induced by non-favorable environmental

conditions (Chalker, 1999). Environmentally induced synthesis is a process that can be taken advantage of in order to produce high quantities of this valued compound. Anthocyanins are known to induced by numerous environmental signals; however one that is generally consistently needed is light stress (Mancinelli, 1985). The application of excessive or specific light has the potential to serve as a mechanism for transforming electromagnetic radiation into high quantities of valuable chemical compounds; i.e. anthocyanins.

To meet demand for anthocyanins, a plant species that could produce high quantities in a sustainable manner is necessary. Turfgrasses are a group of perennial monocots that can withstand constant aggressive harvesting without effecting the growth of new tissues; therefore compounds of interest could be harvested over and over on the same plant(s). Rough bluegrass (RB - *Poa trivialis* L.), a common weed in the turfgrass system, is known to naturally produce a purple color near the crown of the plant (Hurley, 2010). Because of its poor tolerance to abiotic stress, RB is not a widely used turf; however this poor environmental tolerance is what make RB a model species for stress induced anthocyanin production. Cool, wet, and shaded environments provide a well-adapted zone for RB growth (Edminster, 1994). However, when exposed to unfavorable conditions rough bluegrass can produce excess amounts of anthocyanins.

Previous experiments using rough bluegrass (cv. 'Havana') showed that when exposed to prolonged high irradiance white light, anthocyanin concentration could increase by 12,000% or more. When compared to many of the plants currently used as sources of anthocyanins, this species is above average for the total amount of anthocyanins that it can produce (Giusti and Wrolstad, 2001). It is also well known that natural variants within a species can sometimes show relatively high amounts of variation in their ability to accumulate anthocyanins. Kalt et al., 1999 has shown that there is a high amount of variation in total anthocyanin concentration

within species and between closely related species of blueberry (*Cyanococcus* sp.). The same has been seen in red (purple) skinned potatoes (*Solanum tuberosum* L.). Potato cultivars not only showed differences in concentration between cultivars, but differences in anthocyanin concentration could also be seen between growing locations (Lachman et al., 2009). Currently, only rough bluegrass cv. 'Havana' has been tested for its ability to upregulate anthocyanin synthesis; however, there are numerous natural populations of this species across the world along with many other bred cultivars.

In order to plausibly use rough bluegrass as a source of anthocyanins it is necessary to evaluate if this ability is unique to 'Havana', or if it is a property of the entire species. The purpose of this study was to first determine if the ability to up-regulate anthocyanin synthesis is variable among natural RB accessions and bred cultivars, and second to determine what may limit or promote anthocyanin synthesis among these samples.

Materials and Methods

Light emitting diode (LED) array construction

In order to induce the biosynthesis of anthocyanins, a blue light emitting diode (LED) array was constructed as follows. Blue LED's (472nm) were purchased as 5050 surface-mount device strips mounted on metal-core printed circuit boards (Torchstar© www.torchstar.biz). Each strip contained 60 LED's m^{-1}, consuming approximately 72 watts (12 volt DC & 250mA), and producing a beam angle of 120°. LED strips were mounted on a 1.22m x .61m 16 gauge aluminum sheet using .127mm thick 3M 8805 double sided thermally conductive tape (3M Co. Dublin, OH USA).

The final array consisted of 54 LED strips (placed together with no space between) cut at 1.17m, where each strip contained 69 individual LED's. Four-five strips were soldered together

using 16 gauge standard wire and rosin core solder. Each set of wired strips was considered an individual unit that received power at both ends in order to maintain consistent light intensity throughout the strip. Twelve volt constant current LED drivers (400 watt) were used to regulate power to the LED's strips. A total of 8 drivers were used on the array to avoid over-loading a power supply or under-powering LED strips.

The constructed LED array was mounted within a Conviron E15 growth chamber (Controlled Environment Ltd., Winnipeg, Canada) by hanging underneath a pre-existing adjustable light rack using spring loaded carabiners. LED drivers were placed on top of the light rack and wire leads from the LED light strips were fed up the to the power sources to be connected. This setup allowed for precise distance control, where light intensity at the canopy could be tuned by adjusting the distance between the canopy and the LED array. In order to avoid LED overheating, 3 250 CFM ventilation duct blowers were placed on top of the light rack and were allowed to directly circulate air over the LED array.

Plant materials

Accessions and cultivars (Table 1) of RB were obtained from the Western Regional Plant Introduction Station (Washington, USA). Rough bluegrass cv. 'Havana' (I.D. 25) was obtained from Jacklin seed (Jacklin® J.R. Simplot USA). Four replicates of each sample were seeded at a rate of 7.5 g m^{-2} during the month of January (2013) in soilless peat based media and were maintained under greenhouse conditions until experiments were initiated.

All plants were grown in Howlett Hall greenhouse (680 Tharp St. Columbus, OH 43210), The Ohio State University. Leaf tissue was maintained at 7.62 cm by clipping weekly. Plants were hand watered daily, fertilized bi-weekly at a rate of 4.8 g. nitrogen m^{-2} using Peters Professional® 20-10-20 (Scotts LLC, Marysville OH, USA) and periodically treated with

pesticide. Imidacloprid N-[1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl] nitramide (Bayer Environmental Science, Research Triangle PK, NC) was preventively and post-emergently applied for insect control. Mefenoxam (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester (Syngenta AG, Greensboro, NC) and thiophanate-methyl (dimethyl 4,4'-o-phenylenebis[3-thioallophanate]) (Cleary Chemicals, Dayton, NJ) were used for disease control and prevention. Environmental conditions in the greenhouse were setup as follows: 15 hour photoperiod, total light threshold of 300 watt/m^2, daytime cooling = 74°F, daytime heating = 70°F, nighttime cooling = 67°F, and nighttime heating = 63°F.

Light treatment

Five months following seedling germination 4 replicates of each sample were exposed to 5 days of non-intermittent blue light using the previously described LED array. The growth chamber used was set to a constant 15°C for the entire treatment period and plants were watered daily to mitigate drought stress. With 25 samples and 4 replicates each, all samples could not be experimented on at once. Therefore, the experiment was designed as an incomplete block where the experiment was split into 4 groups. Six or 7 samples were randomly chosen and assigned a group, and following the treatment of all 4 groups the experiment was complete. The entire experiment was repeated a 2^{nd} time using the same plants as the previous experiment; therefore, plant material was approximately 7 months old at the initiation of the 2^{nd} experiment. To account for growth chamber variability, the LED array was moved to a second chamber for experiment 2.

Sample Preparation

Following light treatment, all plant material 2.5 cm above the soil surface was harvested. Tissue was directly placed into a No. 6 coin envelope and was subsequently snap frozen in liquid nitrogen, and were then snap frozen all samples were freeze dried for 24 hrs. using a Virtis manifold lyophilizer (The Virtis Co., Gardiner, NY). All samples were then ground to a fine powder using a mortar and pestle and liquid nitrogen. Powdered samples not used for analysis were kept within a -20°C freezer.

Anthocyanin extraction and analysis

Extraction of total anthocyanins and their analysis was performed using the methods of Rodriguez-Saona and Wrolstad (2001). Approximately 50 mg samples of powdered tissue were homogenized in 10 mL of acetone using a Fisher Scientific powergen 500 mechanical homogenizer (Fisher Scientific, Pittsburgh, PA). The resulting sample was centrifuged at 3,600 RPM for 5 min. and the supernatant was collected. The tissue pellet was extracted 2 more times using 5 mL of 70% acetone acidified to 1% with hydrochloric acid. All 3 extracts were combined and were vacuum filtered using Whatman No. 1 filters (Whatman, Kent, United Kingdom). The filtered extract was combined with a 1:1 volume of chloroform, thoroughly vortexted, and was centrifuged at 3,600 RPM for 30 min. at 15°C.

The uppermost layer containing anthocyanins and other water soluble compounds was removed and placed into 1.5 mL microfuge tubes. Remaining acetone in the anthocyanin rich layer was evaporated using a Savant HSC10K centrifugal evaporator. Samples were evaporated at 10,000 RPM for 35 min. under approximately -90 kPa and were kept cool by cold water circulation. Following acetone evaporation, final extract volume was recorded and was used for determination of anthocyanin concentration.

To determine total monomeric anthocyanin concentration the pH differential method was used (Giusti and Wrolstad, 2001). An aliquot of anthocyanin extract was combined in 2 separate buffers where the extract was 20% or less of the final volume. Buffer A consisted of a 0.025 M potassium chloride buffer adjusted to pH 1.0 and buffer B consisted of a 0.4 M sodium acetate buffer adjusted to pH 4.5. After combination with the appropriate buffer, samples were left in the dark for 10-15 min., and were then analyzed spectrophotometrically at wavelengths 510 and 700 nm (Shimadzu UV-1800 - Shimadzu Scientific Instruments Inc., Columbia, MD). Total monomeric anthocyanin concentration was determined by the equations of Giusti and Wrolstad, 2001.

Pre-treatment analysis

Total flavonoid and phenolic analysis

Both soluble flavonoids and phenolics were evaluated prior to light treatment and were extracted using the following method. Approximately 25 mg of freeze dried powdered tissue was combined with 5 mL of methanol and was mechanically homogenized. Samples were thoroughly vortexed, allowed to stand at room temperature for 10 min., and were centrifuged at 3,400 rpm for 5 minutes. The supernatant was then used for analysis of either total phenolics or flavonoids (Pourmorad et al., 2006).

Soluble flavonoids were measured using the method described by Pourmorad et al., (2006). A standard curve was prepared using quercetin solutions at concentrations of 0, 25, 50, 75, 100, and 125 mg L^{-1} in methanol. The extracted supernatant (or quercetin standard) was then combined with 1.5 mL methanol, 0.1 mL of 10% Aluminum chloride, 0.1 mL of 1M potassium acetate, and was diluted with 2.8 mL of distilled water. All samples were vortexed and allowed to stand in the dark at room temperature for 30 minutes. Absorbance was measured at 415 nm,

and concentrations were determined using the quercetin calibration curve (Shimadzu UV-1800 - Shimadzu Scientific Instruments Inc., Columbia, MD).

Total phenolics were measured in accordance with McDonald et al., (2001). Gallic acid standard curves were made using concentrations of 0, 25, 40, 65, 80, and 100 mg L^{-1} solubilized in methanol:water (1:1 v/v). Supernatant previously collected (or gallic acid standard) was combined with 5 mL of 10% folin-ciocalteu reagent and 4 mL of 1M sodium carbonate. Samples were left to sit at room temperature in the dark for 15 minutes. Absorbance was measured at 765 nm, and concentrations were determined using the gallic acid calibration curve (Shimadzu UV-1800 - Shimadzu Scientific Instruments Inc., Columbia, MD).

Total chlorophylls and carotenoids

Both chlorophylls and carotenoids were extracted and analyzed according to Wellburn (1994). Prior to light treatment fresh leaf samples were clipped, measured for area using digital calipers, and were extracted in 5 mL of N,N-Dimethylformamide. Samples were left to sit in the dark at room temperature and allowed to extract for approximately 1 hour. Following this period, samples were refrigerated at $1^{\circ C}$ for 24 – 48 hours. Aliquots of extract were analyzed spectrophotometrically at wavelengths of 663.8, 646.8, and 480 nm (Shimadzu UV-1800 - Shimadzu Scientific Instruments Inc., Columbia, MD). Final concentrations were determined by the equations of Wellburn (1994).

Epicuticular wax extraction and analysis

Total epicuticular waxes were extracted and analyzed prior to light treatment according to the methods Ebercon et al., (1976). Approximately 0.5 g of fresh leaf tissue was extracted for 20 sec. in 20 mL of chloroform. A wax standard curve was developed from carnauba wax solubilized in chloroform and a calibration curve was developed using the following dilutions 0,

6.25, 12.5, 25, 50, 100, 200 mg L^{-1}. Chloroform in either samples or wax standards was boiled off in a hot water bath and the residue was used for further analysis. Samples were placed back on a hot water bath where 5 mL of an acidified potassium dichromate reagent was added and allowed to react for 30 minutes. Samples were allowed to cool and 12 mL of distilled water was added. After cooling for 10-15 min. absorbance at 590 nm was read. Concentration of total epicuticular wax was determined using the carnauba wax standard curve.

Specific leaf area determination

Specific leaf area (SLA - cm^2/mg) is a measure of leaf thickness and is typically measured using single leaves of the same age (Wilson et al., 1999). However, with small bladed turfgrasses this method lacks precision. To make up for this, random leaf samples from across the entire turfgrass canopy were sampled as a means for determining average canopy SLA. Five random leaves were clipped, measured for area, and were oven dried at 55°C for 24 hours. Following drying, samples were weighed and SLA was calculated by dividing leaf area by dry mass (Garnier et al., 2001).

Data Analysis

Data obtained for total monomeric anthocyanin concentration was analyzed as an incomplete block design and was evaluated using SAS 9.2 and the mixed models procedure (SAS Institute, Cary, NC). Classification variables included accession, replicate, experiment (for both experiments 1 and 2) as well as group. Because all 25 samples could not be experimented on at once, they were divided into groups. Each experiment consisted of 4 groups, where following the treatment of all 4 groups the experiment was complete. Principle component analysis was performed using the princomp procedure (SAS 9.2), and correlations were obtained

using the reg procedure. Clustering was performed using R version 3.0.2 (The R Foundation for Statistical Computing) and the Euclidian distance method.

Results

Using pre-treatment data, a cluster analysis was performed to determine how all 25 samples were related. Accessions and cultivars were clustered based on the following variables: phenolics, flavonoids, cuticular waxes, specific leaf area, and chlorophyll $\alpha + \beta$: total carotenoids. Based on the results of the cluster analysis (Figure 1), 5 separate clusters were visually distinct. These clusters could also be defined by the geographic region of samples comprising the cluster. Samples #1-3 all from Afghanistan formed a single cluster, those from Iran again formed a single cluster, and cultivars also made up another distinct cluster. Only 1 sample, # 20, failed to cluster into a logical geographic region; however, this sample still displayed similar attributes to the other samples within it's cluster.

Experiments 1 and 2 showed no statistical differences between one another in terms of total monomeric anthocyanin concentration. Therefore, data was pooled and analyzed together for these two experiments. Ultimately, these results showed that stress induced anthocyanin biosynthesis is common for most ecotypes of RB. However, there are still some accession that even when under a high degree of stress anthocyanin production is limited. Three groups visually stood out when making comparisons using a 95% confidence interval (Table 2). A group producing low concentrations of anthocyanins could be considered statistically different from a group producing high concentrations. However, accessions between these two groups (transition) could not be considered statically different from either the low or the high groupings. Samples within the high group consisted of all production cultivars (#21-25), including 'Havana', as well as samples 7 and 8, both from Germany.

Data obtained from pre-treatment analysis was not used as a tool for direct treatment comparison. Rather these data were used to help determine what may be limiting or increasing anthocyanin biosynthesis among these accessions and cultivars. To help answer these questions, all pre-treatment data was subjected to a principal component analysis (PCA); phenolics (phe), flavonoids (flav), wax (total epicuticular wax), specific leaf area (SLA), and chlorophyll $\alpha + \beta$: total carotenoids (ratio). Results seen within the correlation matrix (Figure 3) show that both flavonoids and waxes exhibit negative correlations (-.41 and -.38 respectively) with induced anthocyanin concentration. Also, flavonoids and phenolics are somewhat correlated (.33), being that flavonoids are derived from phenolic pre-cursors.

Resulting eigen values from the PCA (Figure 3) lean toward 3 principal components. These 3 components (Prin's 1-3) account for 75% of the variation, and components with eigen values below .9 were not considered pertinent for further analysis. Principle component 1 was highly loaded with phenolics (.66), flavonoids (.76), and specific leaf area (-.69). Principle component 2 was highly loaded with total epicuticular waxes (.78), and principle component 3 was highly loaded with chlorophyll $\alpha + \beta$: total carotenoids (.84). Principal component scores for Prin's 1-3 were then used for linear regression analysis where total monomeric anthocyanin concentration was set as the dependent variable. Only principle component 2 showed a significant ($\alpha = .05$) correlation with anthocyanin concentration (Figure 4). Even though the correlation coefficient was relatively low (.28), a p-value of .0064 shows the significance of the relationship between total cuticular waxes and anthocyanins produced under light stress.

Total flavonoid content was also evaluated on a subset of samples 48 hours into light treatment. Results show that samples 20 and 5 significantly increase flavonoid levels compared

to than more than half of the samples (Figure 5); on the other hand, samples 1 and 16 show little increase in flavonoid content compared to the other samples tested.

Discussion

Rough bluegrass cv. 'Havana' was shown to produce excessively high concentrations of anthocyanins under prolonged light stress in previous research. However, the question still remained as to whether or not this was a consistent trait among natural ecoptypes and other cultivars of the species. Rajasekar et al., 2006 showed that among accessions of rough bluegrass there was a high degree of genetic variability (Rajasekar et al., 2006). Results from this study show a similar trend in that some accessions or cultivars have a greater ability to induce higher concentrations of anthocyanins under blue light stress. All production cultivars exhibit the same ability to produce high amounts of anthocyanins when compared to 'Havana'. The same can be said for accessions obtained from Germany. Other than these 7 samples, no other accessions exhibited a consistent ability to excessively upregulate anthocyanin biosynthesis.

Based on the cluster analysis, one might assume that samples from the same geographic region would produce similar amounts of anthocyanins. However, this was not the case. Samples #9, 10, and 12 were able to produce higher concentrations than both #11 and 13. All 5 samples are from Iran, share similar features, yet respond differently under blue light stress. Similar results could be seen with accessions from Afghanistan (#1-3). Both accessions #1 and 2 showed little change in color following treatment, but accession #3 exhibited a slightly stronger response to blue light treatment. Physiological response to stress can be quite variable not only within a species, but also within ecoptypes that are closely related. Many may use this cluster analysis as a means to relate accessions and decrease the overall number of samples. However, in this case even closely related accessions showed variable physiological response; therefore,

it's necessary to evaluate each accession or cultivar individually rather than experiment on similar clusters alone.

Cultivars and accessions that share similar morphological traits with 'Havana' exhibited the ability to drastically increase anthocyanin concentration. On the other hand, accessions possessing distinctly different morphology from 'Havana' tended to produce relatively lower anthocyanin concentrations. This could be visually seen when comparing accessions from Iran or Afghanistan with said cultivars. Iranian and Afghani accessions possessed a very coarse leaf morphology that was more similar to Kentucky bluegrass (*Poa pratensis*) than rough bluegrass. Therefore examining differences in morphology in conjunction with anthocyanin production was a logical step to take.

Results from the PCA showed the relationship between anthocyanin concentration and morphology, namely cuticular waxes. Surface wax accumulation results in increased light scattering and these waxes are essential in helping to protect against absorption of ultra violet light and potentially short wavelength visible light (Pfündel et al., 2006). Accessions of RB that possess a thicker cuticle layer may be perceiving less blue light than those with a thinner cuticle. Ultimately if less blue light is perceived, lower concentrations of anthocyanins will be produced. Because anthocyanins and waxes can fill a similar reflective function it makes sense that waxy accessions show less of an ability to synthesize excessive amounts of anthocyanin (Gould 2004). However, whether or not the increase in cuticle thickness directly regulates anthocyanin production has not been investigated, and it is still unknown if these waxy accessions actually reflect more of the blue light being used to treat.

Overall anthocyanin concentrations from these experiments were relatively low when compared to previous results for 'Havana'. Previous research has shown that fertility, especially

nitrogen, can have a negative impact on anthocyanin biosynthesis. Mori and Sakurai 1994, showed that in strawberry cell culture (*Fragaria* × *ananassa*) anthocyanin synthesis was highly dependent on relatively precise ratios of ammonium and nitrate as well being effected by total nitrogen concentration (Mori and Sakurai, 1994). Rubin et al., 2009 has also shown that nitrate serves as a signal for repressing the transcription of anthocyanin genes (Rubin et al., 2009). There is both visual evidence and molecular evidence that nitrogen can negatively regulate anthocyanin synthesis. Fertilizer rates used in this experiment were relatively high, 5,000 PPM (4.8 g. nitrogen m^{-2}), and the sole nitrogen source was nitrate. In combination with peat based media, soil nitrogen may have also been higher than wanted. Controlling fertility prior to and following stress may be a limiting factor for anthocyanin yield. However, anthocyanin content remained similar for all samples between both experiments. Even if nitrogen effected the outcome of these experiments, the results still show that specific accessions and cultivars have a greater ability to induce anthocyanins.

Another overlooked factor that may have contributed to some of the anthocyanin variation seen between samples is plant density and self-shading. The process of anthocyanin production due to light is dependent on actually perceiving the light; therefore, as the plant canopy ages and increases in density anthocyanin content may decrease. Direct light exposure of the tissue is necessary for pigment production, and cell to cell signaling hasn't been documented for initiation of anthocyanin biosynthesis (Singh et al., 1999). Variation in plant density may have been a contributing factor to some of the differences seen for anthocyanin concentration. Accessions that grow in a more bunch type habit increased density at a faster rate than those that primarily spread by stolons. These factors may have contributed to lower anthocyanin

concentrations in accessions from Afghanistan and Iran, both of which exhibit more of a bunch type growth habit when compared to all other samples.

Rough bluegrass is an ideal species for sustainable stress induced anthocyanin production. These plants have been shown to be able to produce excessive quantities of anthocyanins, harvested, and the process allowed to repeat. Secondly, the amount of variability that exists for anthocyanin biosynthesis in this species lends the ability for breeding a specific variety that can be used for anthocyanin production. A variety that possess a thin cuticle and maintains a plant density that allows for efficient light perception while still allowing maximum anthocyanin yield could be bred using results from this initial research. The ability of turfgrasses to re-grow tissue following aggressive harvesting provides a sustainable system for the production of secondary metabolites. Rough bluegrass provides the perfect example of how an efficient system could be designed to maximize the production of a valuable botanical,

Tables and Figures

I.D.	Accession	Country
1	PI 221908	Afghanistan
2	PI 221915	Afghanistan
3	PI 221951	Afghanistan
4	PI 659652	Czech Republic
5	PI 225826	Denmark
6	W6 2820	Germany
7	W6 28122	Germany
8	W6 28268	Germany
9	PI 227672	Iran
10	PI 227858	Iran
11	PI 229719	Iran
12	PI 251407	Iran
13	PI 380993	Iran
14	PI 254908	Iraq
15	PI 659892	Kyrgyzstan
16	PI 422592	Morocco
17	PI 578852 ‘PO-LIS’	Netherlands
18	PI 250982	Serbia/Montenegro
19	PI 251167	Serbia/Montenegro
20	PI 204484	Turkey
21	PI 537439 ‘LASER’	USA
22	PI 592521 ‘ProAm’	USA
23	PI 594396 ‘SABRE’	USA
24	PI 601315 ‘COLT’	USA
25	Havana	USA

Table 1: Accessions/cultivars of rough bluegrass used in this study, their country of origin, and their associated I.D. numbers

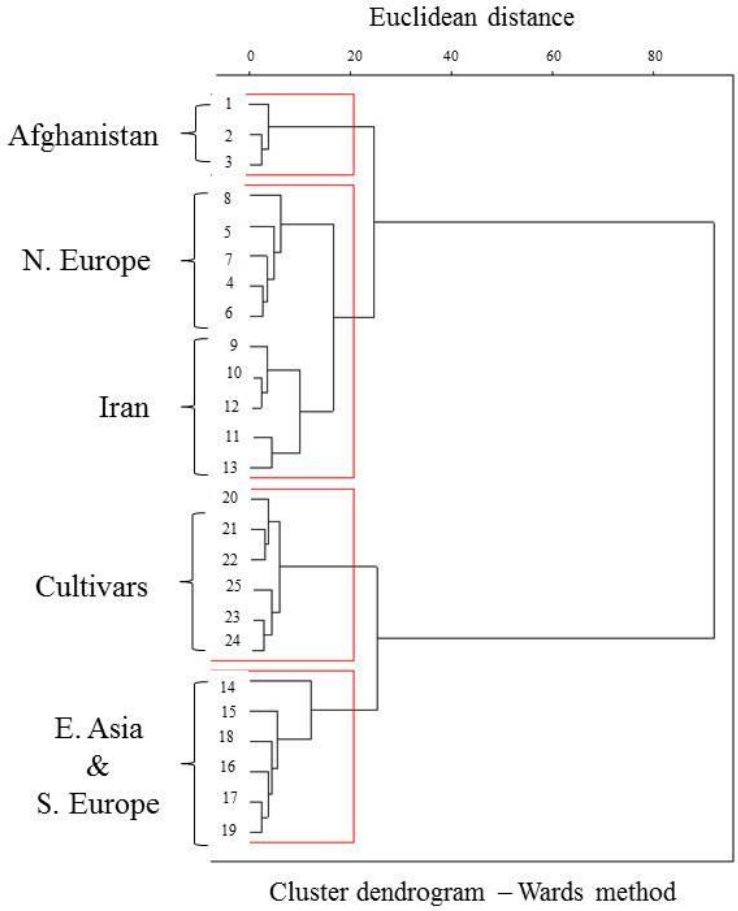


Figure 1: Cluster dendrogram separating accessions and cultivars based on phenolics, flavonoids, cuticular waxes, specific leaf area, and chlorophyll α + β : total carotenoids

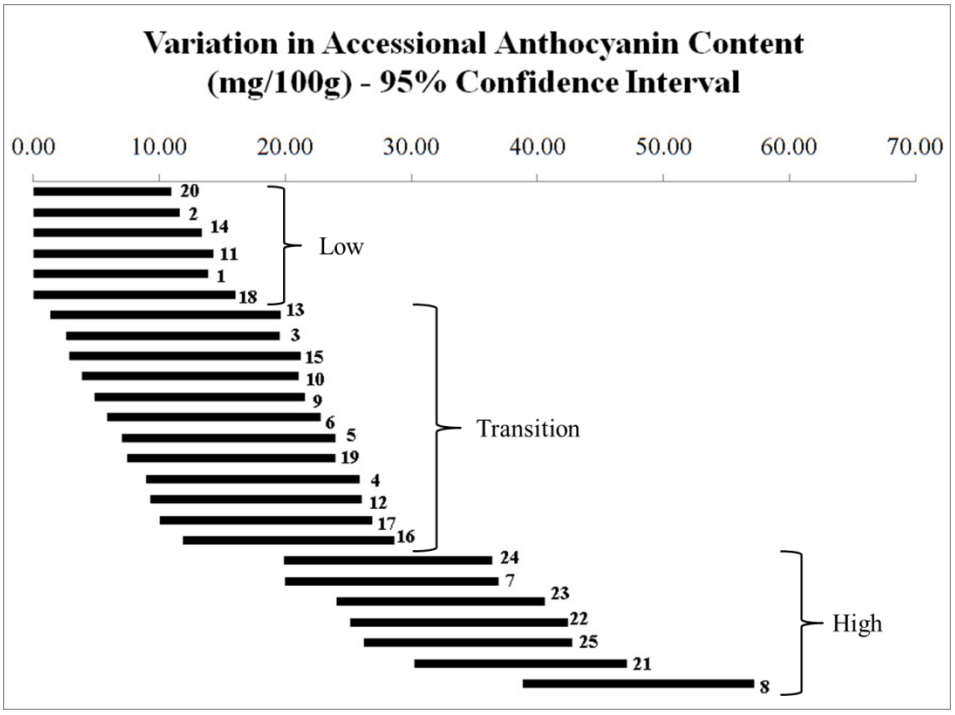


Figure 2: 95% confidence interval separating accessions and cultivars based on blue light induced anthocyanin concentration

Correlation Matrix

	phe	flav	wax	SLA	ratio	antho
phe	1.000	0.327	0.117	-0.186	-0.074	-0.262
flav	0.327	1.000	-0.020	-0.268	-0.093	-0.407
wax	0.117	-0.020	1.000	0.279	-0.099	-0.377
SLA	-0.186	-0.268	0.279	1.000	-0.047	-0.147
ratio	-0.074	-0.093	-0.099	-0.047	1.000	0.255
antho	-0.262	-0.407	-0.377	-0.147	0.255	1.000

Eigen Values of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	1.55	0.28	0.31	0.31
2	1.27	0.34	0.25	0.56
3	0.93	0.28	0.19	0.75
4	0.65	0.05	0.13	0.88
5	0.60	0.12	1.00	

Correlation Coefficients

	Prin 1	Prin 2	Prin 3
phe	0.656	0.406	0.308
flav	0.758	0.186	0.030
wax	-0.218	0.784	0.354
SLA	-0.693	0.429	0.072
ratio	-0.118	-0.526	0.835

Figure 3: Principle component analysis of pre-treatment data

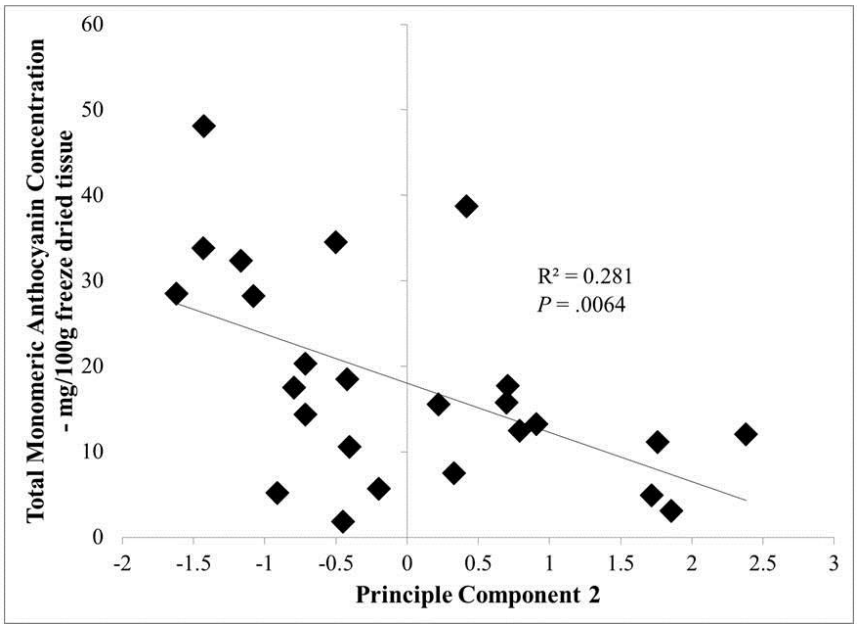
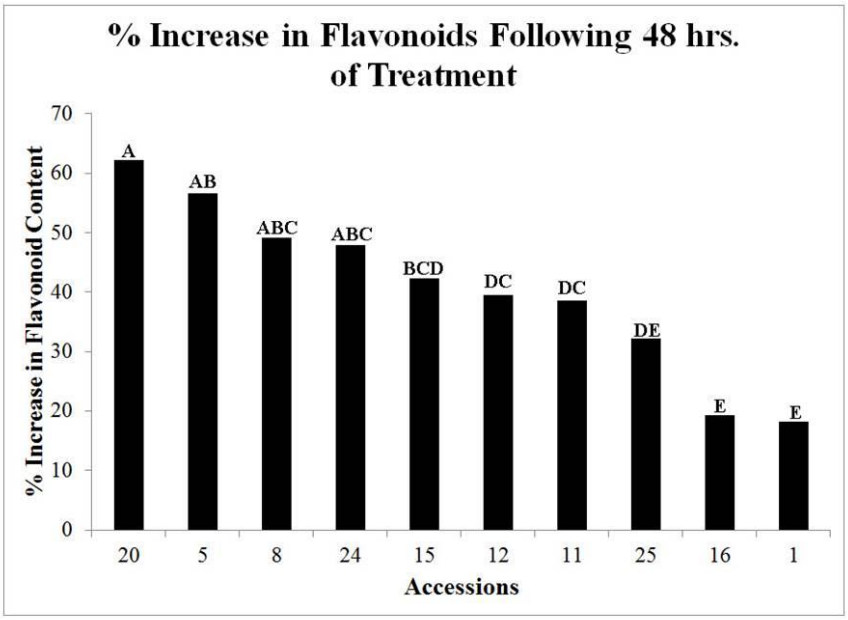


Figure 4: Linear regression of principle component 2 (total cuticular waxes) and associated anthocyanin concentration



LSD = 14.464

Figure 5: Percent increase in total flavonoids following 48 hours of blue light treatment. Bars with same letter are not considered statistically different from one another ($\alpha = .05$)

Refrence

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